JC10 Rec'd PCT/PTO 29 OCT 2001

FORM PTO-1390 (REV. 9-2001) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY 'S DOCKET NUMBER
701826-052090

U.S. APPLICATION NO (If known, see 37 CFR 1.5
To be assigned 10/031540

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/CA00/00482	27 April 2000 (27.04.00)	28 April 1999 (28.04.99)

TITLE OF INVENTION MICROENCAPSULATED GENETICALLY ENGINEERED E. COLI DH 5 CELLS FOR THE REMOVAL OF UNDESIRED ELECTROLYTES AND/OR METABOLITES

APPLICANT(S) FOR DO/EO/US
PRAKASH, Satya and CHANG, Thomas, M., S.,

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [ ] The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   b. [X] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [X] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. [ ] is attached hereto.
   b. [X] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [X] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [X] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
14. [ ] A SECOND or SUBSEQUENT preliminary amendment.
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter.
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. [ ] A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. [X] Other items or information:
    Copy of PCT/IB/308 (1 pg); copy of PCT/IPEA/416 and PCT/IPEA/409 with Amended Sheets (13 pgs); Verified Express Mail Label No. EL565093274US; Checks in the amount of $529 + 165 ; and Return Receipt Postcard; copy of International Search Report (5 pgs).

531 Rec'd PCT/PTO 29 OCT 2001

U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 10/031640 To be assigned	INTERNATIONAL APPLICATION NO PCT/CA00/00482	ATTORNEY'S DOCKET NUMBER 7010826-052090

21. [X] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):

	CALCULATIONS	PTO USE ONLY
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1040.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 890.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [X] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$	
Total claims	16 - 20 =	0	x **$18.00**	$	
Independent claims	5 - 3 =	2	x **$84.00**	$ 168.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$280.00**	$	
			TOTAL OF ABOVE CALCULATIONS =	$ 1188.00	
[X] Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2. +				$ 594.00	
			SUBTOTAL =	$ 594.00	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
			TOTAL NATIONAL FEE =	$ 594.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
			TOTAL FEES ENCLOSED =	$ 594.00	
				Amount to be refunded:	$
				charged:	$

a. [X] A check in the amount of $ 529.00 65.00 - surcharge to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. ______________ in the amount of $ ___________ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-0850. A duplicate copy of this sheet is enclosed.

d. [ ] Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

David S. Resnick
NIXON PEABODY LLP
101 Federal Street
Boston, MA 02110

SIGNATURE

David S. Resnick
NAME

34,235
REGISTRATION NUMBER

Practitioner's Docket No. 701826-052090

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/CA00/00482	27 April 2000 (27.04.00)	28 April 1999 (28.04.99)

TITLE OF INVENTION

MICROENCAPSULATED GENETICALLY ENGINEERED E. COLI DH 5 CELLS FOR THE REMOVAL OF UNDESIRED ELECTROLYTES AND/OR METABOLITES

APPLICANTS

PRAKASH, Satya and CHANG, Thomas M.S.

U.S. SERIAL NO.: 10/031,640

CERTIFICATE OF MAILING

I hereby certify that this correspondence, on the date shown below, is being deposited with the United States Postal Service with sufficient postage as Express Mail Label No. EL565097656US in an envelope addressed to Box PCT, Assistant Commissioner of Patents, Washington, D.C. 20231.

Date: 5/31, 2002

Nicole M. Gignac

Box PCT
Assistant Commissioner for Patents
Washington, D.C. 20231
Attention: DO/US

SECOND PRELIMINARY AMENDMENT

This Preliminary Amendment is being filed in the U.S. Patent and Trademark Office subsequent to the U.S. National Phase Entry of the above-identified application.

Prior to examination on the merits, please amend the application identified in caption as follows:

IN THE SPECIFICATION:

Please insert the following heading and paragraph as the first paragraph on the first page in the application:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a National Phase Entry Application of co-pending International Application PCT/CA00/00482 filed on April 27, 2000 which designated the U.S and which claims priority benefit of U.S. Provisional Application 60/131,468 filed on April 28, 1999.

REMARKS

By the present Preliminary Amendment, Applicant has added the heading and cross-reference information suggested by the U.S. Patent and Trademark Office at the appropriate places in the specification.

In the event that there are any questions relating to this Amendment or to the application in general, it is kindly requested that the Examiner contact the undersigned attorney concerning the same to expedite prosecution of this application.

Entry of the foregoing and prompt and favorable consideration of the subject application on the merits are respectfully requested.

Date: 5/31/02

Customer No.: 26770

Respectfully submitted,

Lana A.

David S. Resnick (Reg. No. 34,235)
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INTERNATIONAL APPLICATION NO.
PCT/CA00/00482

INTERNATIONAL FILING DATE
27 April 2000 (27.04.00)

PRIORITY DATE CLAIMED
28 April 1999 (28.04 99)

VERSION WITH MARKINGS TO SHOW CHANGES MADE TO THE SPECIFICATION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a National Phase Entry Application of co-pending International Application PCT/CA00/00482 filed on April 27, 2000 which designated the U.S and which claims priority benefit of U.S. Provisional Application 60/131,468 filed on April 28, 1999.

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Practitioner's Docket No. 701826-052090

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/CA00/00482	27 April 2000 (27.04.00)	28 April 1999 (28.04.99)

TITLE OF INVENTION

MICROENCAPSULATED GENETICALLY ENGINEERED E. COLI DH 5 CELLS FOR THE REMOVAL OF UNDESIRED ELECTROLYTES AND/OR METABOLITES

APPLICANTS

PRAKASH, Satya and CHANG, Thomas M.S.

U.S. SERIAL NO.: 10/031,640

CERTIFICATE OF MAILING

I hereby certify that this correspondence, on the date shown below, is being deposited with the United States Postal Service with sufficient postage as Express Mail Label No. EL565097656US in an envelope addressed to Box PCT, Assistant Commissioner of Patents, Washington, D.C. 20231.

Date: 5/31, 2002 Nicole M. Gignac

Nicole M. Gignac

Box PCT
Assistant Commissioner for Patents
Washington, D.C. 20231
Attention: DO/US

REQUEST TO MAKE CITATIONS OF RECORD

Applicants respectfully request that the references set forth in the attached PTO/SB08/A and PTO/SB08/B be made of record in this application. Applicants note that the Notification of Missing Requirements mailed April 1, 2002 indicates the copies of the references cited in the International Search Report were received in this application.

FEE AUTHORIZATION

Should any fees associated with the submission be required, the Commissioner is authorized to charge the missing fee to our Deposit Account No. 50-0850.

Date: 5/31/02

Customer No.: 26770

Respectfully submitted,

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10/031640
531 Rec'd PCT/PTC 29 OCT 2001

Practitioner's Docket No. 701826-052090

CHAPTER II

TO THE UNITED STATES ELECTED OFFICE (EO/US)
(ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/CA00/00482	27 April 2000 (27.04.00)	28 April 1999 (28.04.99)

TITLE OF INVENTION

MICROENCAPSULATED GENETICALLY ENGINEERED E. COLI DH 5 CELLS FOR THE REMOVAL OF UNDESIRED ELECTROLYTES AND/OR METABOLITES

APPLICANTS

PRAKASH, Satya and CHANG, Thomas M.

CERTIFICATE OF MAILING

I hereby certify that this correspondence, on the date shown below, is being deposited with the United States Postal Service with sufficient postage as Express Mail in an envelope Label No. EL565093274US addressed to Assistant Commissioner of Patents, Washington, D.C. 20231.

Date: 29 October 2001

Nicole M. Gignac

Nicole M Gignac

Box PCT
Assistant Commissioner for Patents
Washington D.C. 20231

Attention: EO/US

PRELIMINARY AMENDMENT

This Preliminary Amendment is being filed in the U.S. Patent and Trademark Office concurrently with the U.S. National Phase Entry of the above-identified application.

Preliminary to calculation of the filing fee and examination on the merits, please amend the application identified in caption as follows:

IN THE CLAIMS:

Please amend claims 6, 10, 11, 12, 13 and 14 as follows:

6. A method of the treatment of a disease with elevated level of undesired electrolytes and/or metabolites in the body of a patient, which comprises treating said patient with a composition for the removal of at least one undesired electrolyte and/or metabolite, wherein the composition comprises a genetically engineered *E. coli DH5* cells

microencapsulated in artificial cells to be capable of removing said undesired electrolyte and/or metabolite.

10. The method of claim 6, wherein said undesired electrolyte is selected from the group consisting of K, Mg, P, Na, Cl and said undesired metabolite is selected from the group consisting of uric acid, cholesterol, bilirubin, and creatinine, wherein said removal of undesired electrolyte and/or metabolite lowers the undesired chemical concentration to a therapeutically acceptable level.

11. The method of claim 6, wherein said *E. coli DH5* cell is microencapsulated using a microcapsule material which can retain the *E. coli DH5* cells and allows the undesired electrolyte and/or metabolite for removal to enter the microcapsules.

12. The method of claim 6, wherein said *E. coli DH5* cells are entrapped within a carrier using an entrapment material which can retain the cells and allows the undesired electrolyte and/or metabolite for removal to enter in contact with the entrapped cells.

13. The method of claim 11, wherein said *E. coli DH5* cells are microencapsulated using a material selected from the group consisting of nylon, silicon rubber, nylon-polyethylenimine, polylactic acid, polyglycolic acid, chitosan-alginate, cellulosesulphate-poly (dimethyldiallyl)-ammonium chloride, hydroxyethyl methacrylate-methyl methacrylate, chitosan carboxymethyl-cellulose and alginate-polylysinealginate.

14. A method for the *in vitro* removal of at least one undesired electrolyte and/or metabolite in the body of a patient, the method comprising contacting plasma of the patient with genetically engineered *E. coli DH5* cells microencapsulated to be capable of removing said undesired electrolyte is selected from the group consisting of K, Mg, P, Na, Cl and said undesired metabolite is selected from the group consisting of uric acid, cholesterol, bilirubin, and creatinine, wherein said removal of undesired electrolyte and/or metabolite lowers the undesired chemical concentration to a therapeutically acceptable level.

INTERNATIONAL APPLICATION NO. PCT/CA00/00482

INTERNATIONAL FILING DATE 27 April 2000 (27 04.00)

PRIORITY DATE CLAIMED 28 April 1999 (28.04.99)

REMARKS

Claims 6, 10, 11, 12, 13 and 14 have been amended. No new matter has been added by virtue of the amendments to the claims.

In view of the foregoing amendment it is respectfully submitted that all claims are in condition for allowance. Early and favorable action is requested.

If any additional fee is required, charge Deposit Account No. 50-0850.

Date: 29 October 2001

Customer No.: 26770

Respectfully submitted,

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ARTIFICIAL CELLS MICROENCAPSULATED GENETICALLY ENGINEERED *E. COLI DH 5* CELLS FOR THE REMOVAL OF UNDESIRED ELECTROLYTES AND/OR METABOLITES

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates to artificial cells for the removal of at least one undesired electrolyte and/or metabolite in a patient and compositions thereof.

(b) Description of Prior Art

High level of one or more systemic K, Mg, P, Na, Cl, uric acid, bilirubin, cholesterol, and creatinine occurs in a number of diseases. The most common example is in acute or terminal kidney failure resulting in elevation of many of these electrolytes and metabolites. Thus, in acute renal failure, rapid increase in systemic potassium level can cause the death of the patient. In terminal renal failure, K, Mg, P, Na, Cl, uric acid and creatinine need to be lowered. Other examples include bilirubin in liver failure, hyperbilirubinemia and other conditions. Increase in cholesterol is related to arteriosclerosis that can cause cardiovascular diseases and stroke. Uric acid is markedly increased in gout and in other conditions.

At present lowering of these metabolites is done by using dialysis, oral adsorbents and other techniques. Dialysis for kidney failure is expensive and inconvenient. Removal of bilirubin, uric acid, cholesterol etc is difficult.

Therefore, a suitable affordable method to lower these metabolites from the body fluid compartment is required. In earlier studies, Applicants have shown that using the artificial cell microencapsulated genetically engineered *E. coli DH5* cells it is possible

to lower the plasma urea and ammonia effectively both *in vitro* and from renal failure experimental uremic rats (PCT Application published under No. WO 97/26903 on July 31, 1997). However, removing urea and ammonia alone is not enough to treat kidney failure or liver failure respectively.

It would be highly desirable to be provided with a tool for lowering of K, Mg, P, Na, Cl, uric acid, cholesterol, bilirubin, and creatinine in patients.

SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided a composition for the removal of at least one undesired electrolyte and/or metabolite in a patient, which comprises a genetically engineered E. *coli DH5* cells microencapsulated in artificial cells to be capable of removing said undesired electrolyte and/or metabolite, wherein said undesired electrolyte is selected from the group consisting of K, Mg, P, Na, Cl and said undesired metabolite is selected from the group consisting of uric acid, cholesterol, bilirubin, and creatinine, wherein said removal of undesired electrolyte and/or metabolite lowers the undesired chemical concentration to a therapeutically acceptable level.

The microorganism, *E. coli DH5* cells, is microencapsulated using any microcapsule material which can retain the *E. coli DH5* cells and allows the undesired electrolyte and/or metabolite for removal to enter the microcapsules.

The *E. coli DH5* cells are entrapped within a carrier using any entrapment material which can retain the cells and allows the undesired electrolyte and/or

metabolite for removal to enter in contact with the entrapped cells.

The *E. coli DH5* cells are microencapsulated using any material selected from the group consisting of nylon, silicon rubber, nylon-polyethylenimine, polylactic acid, polyglycolic acid, chitosan-alginate, cellulosesulphate-poly(dimethyldiallyl)-ammonium chloride, hydroxy-ethyl methacrylate-methyl methacrylate, chitosan-carboxymethyl-cellulose and alginate-polylysine-alginate.

In accordance with the present invention, there is provided a method of treatment of a disease with elevated level of undesired electrolytes and/or metabolites in plasma of a patient, which comprises treating said patient with a composition of the present invention for the removal of at least one undesired electrolyte and/or metabolite.

The disease may be a kidney failure-causing disease, a liver failure-causing disease or a hyperammonemia with elevated ammonia level.

In accordance with the present invention, there is provided artificial cells for the *in vitro* removal of at least one undesired electrolyte and/or metabolite in plasma of a patient, which comprises genetically engineered *E. coli DH5* cells microencapsulated to be capable of removing said undesired electrolyte and/or metabolite, wherein said undesired electrolyte is selected from the group consisting of K, Mg, P, Na, Cl and said undesired metabolite is selected from the group consisting of uric acid, cholesterol, bilirubin, and creatinine, wherein said removal of undesired electrolyte and/or metabolite lowers the undesired

chemical concentration to a therapeutically acceptable level.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates plasma potassium removal by free genetically engineered *E. coli DH5* cells and APA-membrane artificial cell containing genetically engineered *E. coli DH5* cells;

Fig. 2 illustrates plasma phosphorous removal by free genetically engineered *E. coli DH5* cells and APA-membrane artificial cell containing genetically engineered *E. coli DH5* cells;

Fig. 3 illustrates plasma magnesium removal by free genetically engineered *E. coli DH5* cells and APA-membrane artificial cell containing genetically engineered *E. coli DH5* cells;

Fig. 4 illustrates plasma sodium removal by free genetically engineered *E. coli DH5* cells and APA-membrane artificial cell containing genetically engineered *E. coli DH5* cells;

Fig. 5 illustrates plasma chloride removal by free genetically engineered *E. coli DH5* cells and APA-membrane artificial cell containing genetically engineered *E. coli DH5* cells;

Fig. 6 illustrates plasma cholesterol removal by free genetically engineered *E. coli DH5* cells and APA-membrane artificial cell containing genetically engineered *E. coli DH5* cells;

Fig. 7 illustrates plasma bilirubin removal by free genetically engineered *E. coli DH5* cells and APA-membrane artificial cell containing genetically engineered *E. coli DH5* cells;

Fig. 8 illustrates plasma creatinine removal by free genetically engineered *E. coli DH5* cells and APA-membrane artificial cell containing genetically engineered *E. coli DH5* cells;

Fig. 9 illustrates plasma uric acid removal by free genetically engineered *E. coli DH5* cells and APA-membrane artificial cell containing genetically engineered *E. coli DH5* cells;

Fig. 10 illustrates *in vivo* plasma uric acid removal by oral administration of APA-membrane artificial cell containing genetically engineered *E. coli DH5* cells;

Fig. 10 illustrates *in vivo* plasma uric acid removal by oral administration of APA-membrane artificial cell containing genetically engineered *E. coli DH5* cells;

Fig. 11 illustrates *in vivo* plasma chloride removal by oral administration of APA-membrane artificial cell containing genetically engineered *E. coli DH5* cells;

Fig. 12 illustrates *in vivo* plasma cholesterol removal by oral administration of APA-membrane artificial cell containing genetically engineered *E. coli DH5* cells;

Fig. 13 illustrates *in vivo* plasma creatinine removal by oral administration of APA-membrane artificial cell containing genetically engineered *E. coli DH5* cells;

Fig. 14 illustrates *in vivo* plasma potassium removal by oral administration of APA-membrane artificial cell containing genetically engineered *E. coli DH5* cells; and

Fig. 15 illustrates *in vivo* plasma phosphate removal by oral administration of APA-membrane artificial cell containing genetically engineered *E. coli DH5* cells.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, Applicants reports the use of artificial cells microencapsulated genetically engineered *E. coli DH5* cells for lowering of K, Mg, P, Na, Cl, uric acid, cholesterol, bilirubin, and creatinine in a patient. Result shows that this novel approach has great ability to significantly lower these metabolites from the plasma and has much potential to provide a novel method to the existing system for the purpose.

MATERIALS AND METHODS

Chemicals:

Alginic acid (low viscosity, Lot 611994) and poly-L-lysine (MW 16,100, Lot 11H5516) were purchased from Kelco and Sigma Chemical Co. (St. Louis, MO, USA) respectively. Unless specified, chemicals were obtained commercially and not further purified before use and they were of analytical reagent grade. Uric acid (lot 37H1291, molecular weight 168.10) used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and has the following impurities: Al<0.0005%, Ca<0.01%, Cu<0.0005%, Fe<0.0005%, Mg<0.001%, Na<0.01%, NH4+1<0.05%, P<0.005%, Pb<0.001%, Zn<0.0005%.

Microorganism and Culture Conditions:

Genetically engineered bacteria *Escheretia coli* DH5, containing the urease gene from *Klebsiella aerogens*, was a generous gift from Prof. R. P. Haussinger (Mobley, H. L. and Haussinger, R. P. (1989) *Microbiol. Rev.* 53: pp. 85-108). Luria-Bertani (LB) growth medium was used for primary cell cultivation. The composition of LB medium was of 10.00 g/L bactotryptone (Difco), 5.00 g/L bacto yeast extract (Difco), and 10.00 g/L sodium chloride (Sigma). The pH

was adjusted to 7.5 by adding about 1.00 ml of 1.00 N NaOH. Media were then sterilized in Castle Labclaves for 30 minutes at 250°C. Incubation was carried out in 5.00 ml LB in 16.00 ml culture tubes at 37°C in an orbital shaker at 120 rpm. For the large-scale production of biomass, for microencapsulation purpose, 250 ml Erlenmeyer flask containing 100 ml LB medium was used.

Micro-organism Induction Procedure:

To increase the efficiency of the genetically engineered cells, metabolic fermentation induction was performed. For this genetically engineered *E. coli* DH5 cells were induced by fermentation incubation in a specially designed media called, modified media, which contains a defined chemical compositions for forty six consecutive generations. The media composition was as follows: Potassium mono hydro phosphate 1g/l, Potassium di hydro phosphate 4.0 mg/l, Ammonium sulphate 20 mg/l, Magnesium sulphate septa hydrate 3.4 g/l, Vitamin B1 0.07 g/l, and Trace metal, 5.0 ml. All the media was supplemented with glucose (1g/l) and urea (4 ml form 250 mg/ml stock / 1) filtered) autoclaved in a separate container. This was done in a 250 ml Erlenmeyer flask containing 100 ml of the medium at 37°C in an orbital shaker at 120 rpm.

Microencapsulation Procedure:

The details of microencapsulation procedures are as follows. Microcapsule containing bacterium *E. coli* DH5 cells were prepared as follow: Bacterial cells were suspended in an autoclaved sodium alginate in 0.9 % sodium chloride solution. The viscous alginate-bacterial suspension was pressed through a 23 gauge

needle using a syringe pump (Compact Infusion Pump Model 975, Harvard App. Co. MA). Compressed air was passed through a 16 gauge needle to shear the droplets coming out of the tip of the 23 gauge in a droplet needle. The droplets were allowed to gel for 15 minutes in a gently stirred ice-cold solution of calcium chloride (1.4 %). After gelation in the calcium chloride, alginate gel beads were coated with polylysine (0.05 % in HEPES buffer saline, pH 7.20) for 10 minutes. The beads were then washed with HEPES and coated with an alginate solution (0.1 %) for 4.00 minutes. The alginate-poly-L-lysine-alginate capsules were then washed in a 3.00 % citrate bath (3.00 % in 1:1 HEPES-buffer saline, pH 7.20) to liquefy the gel in the microcapsules. The microcapsules formed were stored at 4°C and used for the experiments.

Microcapsule Storage Condition:

After the microencapsulation microcapsules were washed properly several times (two to three times) with sterile water. The microcapsules were resuspended in the Aggrobacteriium minimum broth (AG minimal media) at 4-10°C. This media, unlike L. B. media, does not support the growth of *E. coli*, it has however all the components which is necessary to maintain biochemical activity (Chang, T.M.S. (1964) *Science* **146**:524-525). Before the use microcapsules were washed in normal saline to remove the media component from the surface and used for the experiment.

Plasma Used:

For all the studies freshly isolated non heparinized plasma from male Whister rats of 170-370 g weight range were used otherwise mentioned.

***In vitro* Experimental Procedure:**

The bacteria were grown in L B medium. Log phase bacterial cells were harvested by centrifuging at 10,000 g for 20 min. at 4°C. The cell mass was then washed five times with sterile cold water to remove media components. Cells were then weighed and used for the plasma K, Mg, P, bilirubin, uric acid and Creatinine removal studies by free genetically engineered *E. coli DH5* cells.

For the microencapsulated *E. coli DH5 in vitro* removal of plasma K, Mg, P, and bilirubin, uric acid and Cretinine studies, equivalent masses of the cells were microencapsulated in APA membrane and used otherwise mentioned. Uremic rat plasma from different uremic rats were isolated and mixed together to make plasma pool before using them for plasma K, Mg, P, and bilirubin removal studies by free and microencapsuletd bacteria removal studies.

For the microencapsulated *E. coli DH5* uric acid and Creatinine removal studies, the equivalent masses of the cells were microencapsulated in APA membrane and used.

For the plasma uric acid and Creatinine removal studies, we used heparin free normal rat plasma with added uric acid and Creatinine from out side. In all the experiment, the ratio of the volume of the plasma used to the amount of microencapsulated bacteria used was held constant.

In all *in vitro* studies, reactions were performed in 50 ml Erlenmeyer flasks at 30°C and 100 rpm, unless otherwise mentioned. The Lab-Line orbital Environ-Shaker equipped with thermal control and air quality was used for this purpose. Sampling was carried out aseptically at designated times. Bacterial cells, in the free bacteria removal studies, were removed from the sample by centrifugation at 15,000 rpm

for 10 minutes at 4°C and supernatant analyzed. The samples were stored at 4°C for suitable amount of time prior to analysis.

Surgical Experimental Rat Model

The surgical procedure for making the uremic rat model involved two steps, one to perform right nephrectomy and the other to ligate the left artery, vein, and ureter, was designed. Male Wister rats of 300-340 g weight range were used. The details of these two steps are as follows:

Step 1: Unilateral (Right) Nephrectomy

The anesthetized animal was placed in ventral recombency with its tail towards the surgeon. The hair in the right dorsal lumbar area was clipped and the skin was swabbed thoroughly with a surgical scrub. A 2-3 cm incision was made into the skin caudal to the rib cage on the right side of the animal. A 2-3 cm incision was then made into the underlying muscle wall. The kidney was pulled through the muscle wall; the renal artery, vein and ureter were then ligated and the kidney was removed by incising the vessels and ureter between the kidney. The ligature remaining tissue was returned to the peritoneal cavity and the muscle wall was sutured. The remaining tissue was returned to the peritoneal cavity and the muscle wall was sutured. The skin incision was closed using 2-3 wound clips.

Step 2: Left Renal Artery / Vein / Ureter / Ligation

The left side of the rat was prepared as if to perform a left nephrectomy. After an incision (2-3 cm) was made in the muscle wall, the left renal artery, vein, and ureter were located. Using a blunt forceps, the left renal vessels and ureter were isolated and separated from the peritoneal connective tissue. The

renal vessels and ureter were ligated using sterile silk suture. The muscle wall was sutured. The skin incision was closed with 2-3 metal wound clips.

***In vivo* Experimental Procedure**

The bacteria were grown in L. B. medium to their log phase and harvested by centrifugation at 10,000 g for 20 min. at 4°C. The cell mass was then washed 5 times with sterile cold water to remove media components. Cells were then weighed and used for removal studies. For the microencapsulated uric acid removal studies an equivalent mass of the cells were microencapsulated and used. For the microencapsulated *in vivo* animal studies, microcapsules containing log phase bacteria were first suspended in 0.8-1.0 ml sterile normal saline (0.9%) in a 5 ml syringe. The floating microcapsules were then administered orally to the experimental rats using a curved 12G-3 1/2 stainless steel gastric lavage tube. Blood sampling was done from the rat after sedating the animals using appropriate amounts of drugs that have been reported not to have any side effects on renal or hepatic functions. The drugs used were atravet (acepromazine) and ketaset (ketamine) in concentrations of 75 mg/kg and 5-10 mg/9 kg intramuscularly, respectively. Blood was withdrawn using a small 23 G1 precision Glide needle from leg artery. Blood samples were then centrifuged immediately in an Eppendroff micro-centrifuge at 4°C and plasma was collected and analyzed for plasma uric acid concentrations.

Plasma K, P, Mg , Na, Cl, Bilirubin, and Cholesterol Determination:

For the determination of plasma K, Mg, P, Na, Cl, Bilirubin and Cholesterol suitable amount of the sample were withdrawn keeping the reaction condition

sterile using a U.V. sterile chamber. The bacterial cells and microcapsule were removed from the sample immediately by centrifugation at 15,000 rpm for 10 minutes at 4°C and the sample were then stored at stored at 4°C for the analysis. The analysis of plasma K, Mg, P, Cl, Na, bilirubin, and cholesterol was carried out at McGill university animal center biochemical, toxicology and immunology analysis lab. The analysis was done using Reflotron from Manheim Boehringer. This Reflotron system is based on dry chemistry and uses fiber optics in its operation.

Plasma Uric Acid Determination:

The concentration of uric acid were determined based on quantitative measurements using the Sigma diagnostics kits product number 686 purchased from Sigma Chemical Co. USA. This kit is for quantitative enzymatic determination of uric acid in serum or plasma at 520 nm. Two enzymes, uricase and peroxidase,are involved in the reaction of this test procedure. Enzyme uricase catalyses the oxidation of uric acid to allantoin, carbon dioxide, and hydrogen peroxide. In the presence of enzyme peroxidase, the hydrogen peroxide formed reacts with 4-aminoantipyrine dye (4-APP) and 3,5-dichloro-2-hydroxybenene at sulfonate (DHBS) to form a quinoeimine dye with an absorbency maximum at 540 nm. The intensity of the colour produced is directly proportional to the uric acid concentration in the sample.

Plasma Creatinine Determination:

The concentration of Creatinine were determined using the Sigma diagnostics kits product number 555 purchased from Sigma Chemical Co. USA. This method is for a quantitative colorimetric determination of

Creatinine in serum, plasma, and urine at 500 nm optical density.

RESULTS:

Experiments were designed to evaluate the use of microencapsulated genetically engineered cell for the removal of uric acid. For the experiment plasma from six different rat weight range from 170g to 370g were isolated, without using any heparin, and mixed. The isolated plasma then divided into two groups as pool of the plasma source for entire plasma *in vitro* studies. To one group uric acid were added from outside and the other group was used as control plasma, with no added uric acid. The concentration of uric acid in the control pool was found to be 5.99 ± 0.62 mg/dl. The addition of uric acid to the plasma resulted in increased plasma uric acid level, the plasma uric acid concentration went up to 88.88 ± 4.63 mg/dl from 5.99 ± 0.62 mg/dl.

Lowering of Plasma Potassium:

Experiments were designed to evaluate the use of microencapsulated genetically engineered cell for the removal of plasma potassium *in vitro*. Results (Fig. 1) shows that both free *E. coli DH5* cells and artificial cell microencapsulated *E. coli DH5* cells were able to lower plasma potassium. Free bacteria were able to lower plasma potassium from 4.37 ± 0.76 mEq/l to 3.63 ± 0.90 mEq/l and APA encapsulated from 5.80± 0.40 mEq/l to 3.50 ± 0.03 mEq/l in 24 hours. Result also shows that the removal of plasma K by free bacteria and encapsulated bacteria is similar (Fig. 1).

Lowering of Plasma Phosphorous

To evaluate the use of microencapsulated genetically engineered cell for the removal of plasma phosphorous *in vitro*. Results (Fig. 2) shows that both

free *E. coli DH5* cells and artificial cell microencapsulated *E. coli DH5* cells were able to lower plasma potassium, experiment were designed. Free *E. coli DH5* cells were able to lower plasma phosphate from 3.31 ± 0.016 mg/dl to 1.20 ± 0.02 mg/dl and APA encapsulated from 2.20± 0.9 mg/dl to 1.49 ± 0.03 mg/dl in 24 hours. Result (Fig. 2) also shows that free bacteria have higher capacity in terms of overall P lowering than encapsulated bacteria. Also it is found that empty microcapsule were also able to lower plasma P however there was statistically significant different was observed when the result was compared with that of microcapsules having genetically engineered *E. coli DH 5* cells (Fig. 2).

Lowering of Plasma Magnesium:

Results (Fig. 3) shows that both free *E. coli DH5* cells and artificial cell microencapsulated *E. coli DH5* cells were able to lower plasma magnesium *in vitro.* Free *E. coli DH5* cells were able to lower plasma magnesium from 0.84 ± mg/dl to 0.74 ± mg/dl and APA encapsulated *E. coli DH5* cells from 0.90± mg/dl to 0.66 ± mg/dl in 24 hours.

Lowering of Plasma Sodium:

Experiment was design to evaluate the plasma sodium removal efficiency of encapsulated and free *E. coli DH5* cells. Result shows that (Fig. 4) both free bacteria and encapsulated bacteria were able to lower the plasma sodium. Free bacteria were able to lower plasma Na from 175 ± 10.24 mEq/l to 132 ± 5.80 mEq/l and encapsulated bacteria was able to lower plasma Na from 172 ± 11.00 mEq/l to 129 ± 6.12 mEq/l in 24 hours (Fig. 4).

Lowering of Plasma Chloride:

Plasma chloride concentration was determined after challenging the plasma with free *E. coli DH 5* cells and encapsulated *E. coli DH 5* cells. Result (Fig. 5) shows that free bacteria were able to plasma chloride concentration from 137 ± 10.10 mEq/l to 107 ± 5.08 mEq/l and encapsulated bacteria were able to lower plasma chloride from 137 ± 6.60 mEq/l to 107 ± 2.00 mEq/l in 24 hours (Fig. 5). Result also shows (Fig. 5) that both free and encapsulated have identical efficiency for plasma chloride removal.

Lowering of Plasma Cholesterol:

Experiments were design to evaluate the plasma cholesterol lowering capacity of free and encapsulated genetically engineered *E. Coli DH5* cell. Result (Fig. 6) shows that both free and encapsulated bacteria were able to lower plasma cholesterol. Free bacteria were able to lower plasma cholesterol from 1.82 ± 0.13 mmol/l to 1.13 ± 0.04 mmol/l and encapsulated bacteria were able to lower plasma cholesterol from 1.86 ± 0.10 mmol/l to 1.37 ± 0.06 mmol/l in 24 hours. The plasma cholesterol removal capacity of encapsulated bacteria, however, found smaller when compared with free bacteria (Fig. 6).

Lowering of Plasma Bilirubin:

Results (Fig. 7) shows that both free *E. coli DH5* cells and artificial cell microencapsulated *E. coli DH5* cells were able to lower plasma magnesium *in vitro*. Free *E. coli DH5* cells were able to lower plasma bilirubin from 6.0 ± 0.20 mg/dl to 3.0 ± 0.21 mg/dl and APA encapsulated *E. coli DH5* cells from 6.00 ± 0.80 mg/dl to 4.00 ± 0.20 mg/dl in 24 hours (Fig. 7).

Lowering of Plasma Creatinine:

Experiments were design to evaluate the plasma Creatinine removal efficiency of the free and encapsulated *E. coli DH 5* cells. Result (Fig. 8) shows that when challenged, 80.21 ± 1.00% of plasma Creatinine was remaining in the case of free bacteria after 24 hours of incubation and 83.31 ± 2.40% plasma Creatinine was remaining after 24 hours of incubation in the case of encapsulated bacteria (Fig. 8).

Lowering of Plasma Uric Acid:

Experiments were designed to evaluate the use of microencapsulated genetically engineered cell for the removal of uric acid. For the experiment plasma from six different rat weight range from 170 g to 370 g were isolated, without using any heparin, and mixed. The isolated plasma then divided into two groups as pool of the plasma source for entire plasma *in vitro* studies. To one group uric acid were added from outside and the other group was used as control plasma, with no added uric acid. The concentration of uric acid in the control pool was found to be 5.99 ± 0.62 mg/dl. The addition of uric acid to the plasma resulted in increased plasma uric acid level, the plasma uric acid concentration went up to 88.88 ± 4.63 mg/ dl from 5.99 ± 0.62 mg/dl.

The experiment were designed to evaluate the plasma uric acid removal capacity of the free genetically engineered *E. coli DH5* cell by adding the log phase L B grown bacterial cells. Also a control was kept using the uric acid pool plasma. The obtained results shows (Fig. 9) that free bacteria were able to plasma *in vitro*. The plasma uric acid level decreased to 3.44 ± 0.16 from 84.80±2.80 mg/dl in 24 hours. In the control experimental group, the plasma uric acid

concentration was fairly steady throughout the experiment.

Experiment were design to evaluate if the artificial cell encapsulated genetically engineered bacteria *E. coli DH5* is capable of lowering the plasma uric acid *in vitro*. Results are shown in Figure 9 shows that that APA encapsulated genetically engineered *E. coli DH5* cells were able to lower plasma uric acid from 84.80 $\pm$ 3.40 mg/ dl to 8.80 $\pm$ 3.12 mg/ dl in 24 hours.

CONCLUSIONS AND SUMMARY:

High level of one or more systemic K, Mg, P, Na, Cl, uric acid, bilirubin, cholesterol, and creatinine occurs in a number of diseases. The most common example is in acute or terminal kidney failure resulting in elevation of many of these electrolytes and metabolites. Thus, in acute renal failure, rapid increase in systemic potassium level can cause the death of the patient. In terminal renal failure, K, Mg, P, Na, Cl, uric acid and creatinine need to be lowered. In the present novel approach, all these electrolytes and metabolites can be removed effectively by encapsulated E.coli DH5 cells. Based on the result obtained the levels of the electrolytes are lowered to a save level. This novel approach can also remove bilirubin and has potential for use in liver failure, hyperbilirubinemia and other conditions. The ability to remove cholesterol has potentials for use in lowering cholesterol is related to arteriosclerosis that can cause cardiovascular diseases and stroke. This approach can very effectively lower uric acid and it may have much potential in lowering uric acid in gout and in other conditions. These approaches may supplement or replace the expensive and inconvenient treatment using dialysis, plasmapheresis, oral adsorbents and medications.

The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

EXAMPLE I

In vitro plasma unwanted metabolite removal efficiency of the artificial cells containing genetically engineered *E. coli DH 5* cells

Metabolite	0 hours of the incubation	After 24 hours of incubation	% lowered
Potassium (mEq/l)	5.80 ± 0.40	3.50 ± 0.03	39.65
Magnesium (mg/dl)	0.90± 0.06	0.66 ± 0.07	26.66
Sodium (mEq/l)	172 ± 11.00	129 ± 6.12	25.00
Phosphorous(mg/dl)	2.20± 0.9	1.49 ± 0.03	32.27
Chloride (mEq/l)	137 ± 6.60	107 ± 2.10	28.03
Uric Acid mg/ dl	84.80 ± 3.40	8.80 ± 3.12	89.69
Bilirubin (mg/dl)	6.00 ± 0.80	4.00 ± 0.20	33.33
Creatinine (mg/dl)	21.40 ± 1.80	17.83 ± 0.80	16.79
Cholesterol (mmol/l)	1.86 ± 0.10	1.37 ± 0.06	26.34

Example II

Lowering of high plasma uric acid levels in experimental rats by oral administration of artificial cell microencapsulated genetically engineered *E. coli DH5* cells

Microcapsules containing genetically engineered bacteria *E. coli* DH5 cells were prepared as described before. Male Wister rats of 300-325 g weight range were used. The experimental surgical model has a high level of plasma uric acid when compared to normal rats (Fig. 10). A suitable quantity of encapsulated bacteria was given daily to each rat. For this purpose microcapsules were first suspended in 0.8-1.0 ml sterile saline in a 5.0 ml syringe and then administered orally using a 12

G gastric lavage tube. Besides monitoring pretreatment uric acid levels in experimental rat as internal control, we also used a control group. The control group receives empty microcapsule containing no bacteria.

Experiments were designed to evaluate the efficiency of encapsulated genetically engineered *E. coli DH5* cells for lowering plasma uric acid by its oral administration. For this two groups of uremic experimental rat on normal rat chaw were selected. One group that receive empty microcapsule and the other group that receives microcapsule containing 1.00 $\pm$ 0.15 mg/g bodyweight of genetically engineered *E. coli DH5* cells. We followed the plasma uric acid concentration of both the groups for 7 days before giving any type of microcapsules. On day 7 we started oral administration of empty microcapsules and the microcapsule containing genetically engineered *E. coli DH5* cells to the respective group and followed their plasma uric acid concentration. Results (Fig. 10) show that the encapsulated bacteria were able to lower the plasma uric acid concentration very efficiently. Encapsulated bacteria were able to lower plasma uric acid concentration from 88.66 $\pm$ 23.67 to 20.33$\pm$ 17.43 mm/L 2 days later (Fig. 10). The control uremic rat group the plasma uric acid concentration remained high at 72.00 $\pm$ 12.01 mm/L on day 1, 79.00$\pm$ 27.83 mm/L on day 4. By continued daily oral administration of the encapsulated *E. coli DH5* cells, the plasma uric acid concentration of nephrectomy induced uremic rats to this normal level for the entire test period. With discontinuation of oral treatment, the plasma uric acid level quickly returned to the high level. Plasma uric acid level went back to 64.67 $\pm$26.27 mm/L, on the very next day followed by 48.00 $\pm$ 25.23 mm/L, 45.33 $\pm$ 6.35 mm/L,

41.33 ± 12.43 mm/L, 59.00 ±19.00 mm/L, 43.34 ± 5.68 mm/L on days 2,3,4,5,6, and day 7, respectively (Fig. 10).

The obtained result shows that this biotechnological approach of using artificial cells microcapsules containing genetically engineered *E. coli DH5* cells *in vitro* has shown very strong potential to be useful for plasma uric acid lowering in various situations. When given orally, the microorganisms will remain immobilized inside the microcapsules. The microcapsules remain intact as they pass down the gastrointestinal tract. Finally, they are excreted intact with the stool in about 24 hours. The membranes of the intact microcapsules are permeable to smaller molecules like uric acid, urea, ammonia, phosphate, etc. Thus, during the passage of the intact microcapsules through the intestine smaller molecules can diffuse into the microcapsules.

We have also evaluated the other unwanted plasma metabolite removal capacity of artificial cell microencapsulated genetically engineered *E. coli DH5* cells *in vivo*. The plasma chloride from 170 ± 17.03 mmol/L to 150.66 ± 31.97 mmol/L on day 2, plasma cholesterol from 2.24 ± 0.2816 mmol/L to 2.30 ± 0.3464 mmol/L on day 2, alkaline phosphatase from 198.33 ± 23.50 to 149.00 ± 21.93 U/L, creatinine from 34.52 ± 5.29 to mm/L 33.00 ± 2.0 mm/L on day 2, potassium from 5.70 ± 0.96 mm/L to 5.62 ± 0.450 mm/L, and the plasma phosphate from 2.57 ± 0.26 mmol/L to 2.41 ± 0.37 mmol/L on the day 2 of the oral administration.

Example III

Lowering of plasma electrolytes and metabolites in experimental rats by oral administration of artificial cell microencapsulated genetically engineered *E. coli DH5* cells

Microcapsules containing genetically engineered bacteria *E. coli* DH5 cells were prepared as described before. Male Wister rats of 300-325g weight range were used. Throughout the control and treatment periods the experimental rats received normal rat chow. During the treatment, a suitable quantity of encapsulated bacteria was given daily to each rat. For this purpose microcapsules were first suspended in 0.8-1.0 ml sterile saline in a 5.0 ml syringe and then administered orally using a 12 G gastric lavage tube. The animal group receiving empty microcapsule containing no bacteria was treated as other control. A quantity of 1.0 $\pm$ 0.15 mg/g body weight of log phase genetically engineered bacteria *E. coli* DH5 cells in microcapsules was administered daily to a group of 43 day old experimental rats. We followed the plasma electrolytes (Sodium, Potassium, Phosphate, Chloride) and metabolites (creatinine, cholesterol, bilirubin, uric acid) concentration of normal and experimental uremic rats for 27 days.

Experiments were designed to evaluate the efficiency of encapsulated genetically engineered *E. coli DH5* cells for lowering plasma electrolytes and metabolites. For this two groups of uremic experimental rat were selected. One group that receive empty microcapsule and the other group that receives microcapsule containing genetically engineered *E. coli DH5* cells. We followed the plasma concentration of both the groups for 7 days before giving any type of the microcapsule. On the day 7 we started oral

administration of empty microcapsules and the microcapsule containing genetically engineered *E. coli DH5* cells to the respective group of the experimental animals and followed their plasma concentration. Results in the Figs. 10-15 show that the encapsulated bacteria were able to lower the plasma concentration of Sodium, Potassium, Phosphate, Chloride, creatinine, cholesterol, bilirubin, and uric acid. By continued daily oral administration maintained the plasma concentration of nephrectomy induced uremic rats to this lowered level for the entire test period. With discontinuation of oral treatment, the plasma level of these electrolytes and metabolites increased to its pretreated high levels.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A composition for the removal of at least one undesired electrolyte and/or metabolite in a patient, which comprises metabolically induced genetically engineered *E. coli DH5* cells microencapsulated in artificial cells to be capable of removing said undesired electrolyte and/or metabolite, wherein said undesired electrolyte is selected from the group consisting of K, Mg, P, Na, Cl and said undesired metabolite is selected from the group consisting of uric acid, cholesterol, bilirubin, and creatinine, wherein said removal of undesired electrolyte and/or metabolite lowers the undesired chemical concentration to a therapeutically acceptable level.

2. The composition of claim 1, wherein said *E. coli DH5* cell is microencapsulated using any microcapsule material which can retain the *E. coli DH5* cells and allows the undesired electrolyte and/or metabolite for removal to enter the microcapsules.

3. The composition of claim 1, wherein said *E. coli DH5* cells are entrapped within a carrier using any entrapment material which can retain the cells and allows the undesired electrolyte and/or metabolite for removal to enter in contact with the entrapped cells.

4. The composition of claim 2, wherein said *E. coli DH5* cells are microencapsulated using any material selected from the group consisting of nylon, silicon rubber, nylon-polyethylenimine, polylactic acid, polyglycolic acid, chitosan-alginate, cellulosesulphate-

poly(dimethyldiallyl)-ammonium chloride, hydroxy-ethyl methacrylate-methyl methacrylate, chitosan-carboxymethyl-cellulose and alginate-polylysine-alginate.

5. The composition of claim 1, wherein said genetically engineered *E. coli DH5* cells are metabolically induced by fermentation induction.

6. A method of treatment of a disease with elevated level of undesired electrolytes and/or metabolites in the body of a patient, which comprises treating said patient with a composition according to claim 1 for the removal of at least one undesired electrolyte and/or metabolite.

7. The method of treatment of claim 6, wherein said disease is a kidney failure-causing disease.

8. The method of treatment of claim 6, wherein said disease is a liver failure-causing disease.

9. The method of treatment of claim 6, wherein said disease is a hyperammonemia with elevated ammonia level.

10. The use of a composition comprising genetically engineered *E. coli DH5* cells microencapsulated in artificial cells for the removal of at least one undesired electrolyte and/or metabolite in a patient, wherein said undesired electrolyte is selected from the group consisting of K, Mg, P, Na, Cl and said undesired metabolite is selected from the group consisting of uric acid, cholesterol, bilirubin, and creatinine, wherein said removal of undesired electrolyte and/or metabolite lowers the undesired chemical concentration to a therapeutically acceptable level.

11. The use of claim 10, wherein said *E. coli DH5* cell is microencapsulated using any microcapsule material which can retain the *E. coli DH5* cells and allows the undesired electrolyte and/or metabolite for removal to enter the microcapsules.

12. The use of claim 10, wherein said *E. coli DH5* cells are entrapped within a carrier using any entrapment material which can retain the cells and allows the undesired electrolyte and/or metabolite for removal to enter in contact with the entrapped cells.

13. The use of claim 11, wherein said *E.coli DH5* cells are microencapsulated using any material selected from the group consisting of nylon, silicon rubber, nylon-polyethylenimine, polylactic acid, polyglycolic acid, chitosan-alginate, cellulosesulphate-poly(dimethyldiallyl)-ammonium chloride, hydroxy-ethyl methacrylate-methyl methacrylate, chitosan-carboxymethyl-cellulose and alginate-polylysine-alginate.

14. The use of artificial cells for the *in vitro* removal of at least one undesired electrolyte and/or metabolite in the body of a patient, which comprises genetically engineered *E. coli DH5* cells microencapsulated to be capable of removing said undesired electrolyte and/or metabolite, wherein said undesired electrolyte is selected from the group consisting of K, Mg, P, Na, Cl and said undesired metabolite is selected from the group consisting of uric acid, cholesterol, bilirubin and creatinine, wherein said removal of undesired electrolyte and/or metabolite lowers the undesired chemical concentration to a therapeutically acceptable level.

15. Artificial cells for the *in vitro* removal of at least one undesired electrolyte and/or metabolite in plasma of a patient, which comprises metabolically induced genetically engineered *E. coli DH5* cells microencapsulated to be capable of removing said undesired electrolyte and/or metabolite, wherein said undesired electrolyte is selected from the group consisting of K, Mg, P, Na, Cl and said undesired metabolite is selected from the group consisting of uric acid, cholesterol, bilirubin and creatinine, wherein said removal of undesired electrolyte and/or metabolite lowers the undesired chemical concentration to a therapeutically acceptable level.

16. The artificial cells of claim 15, wherein said genetically engineered *E. coli DH5* cells are induced by fermentation induction.

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(54) Title: ARTIFICIAL CELLS MICROENCAPSULATED GENETICALLY ENGINEERED *E. COLI DH 5* CELLS FOR THE REMOVAL OF UNDESIRED ELECTROLYTES AND/OR METABOLITES

(57) Abstract

The present invention relates to a composition for the removal of at least one undesired electrolyte and/or metabolite in a patient, which comprises a genetically engineered *E. coli DH5* cells microencapsulated in artificial cells to be capable of removing said undesired electrolyte and/or metabolite, wherein said undesired electrolyte is selected from the group consisting of K, Mg, P, Na, Cl and said undesired metabolite is selected from the group consisting of uric acid, cholesterol, bilirubin, and creatinine, wherein said removal of undesired electrolyte and/or metabolite lowers the undesired chemical concentration to a therapeutically acceptable level.

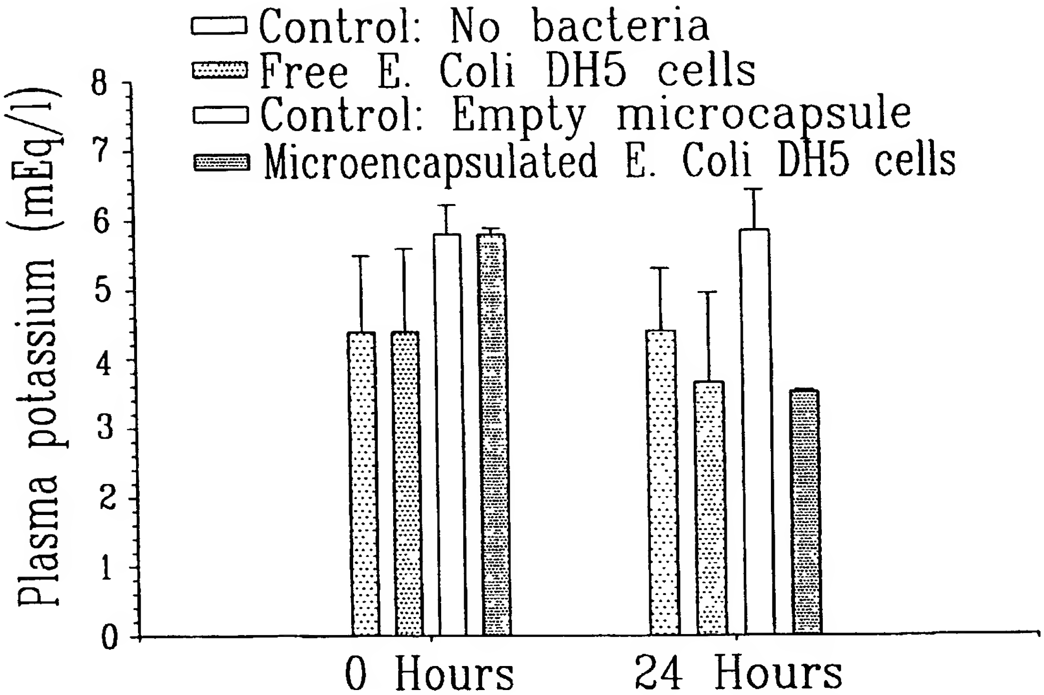

Control: No bacteria
Free E. Coli DH5 cells
Control: Empty microcapsule
Microencapsulated E. Coli DH5 cells
Plasma potassium (mEq/l)
8
7
6
5
4
3
2
1
0
0 Hours
24 Hours

FIG. 1

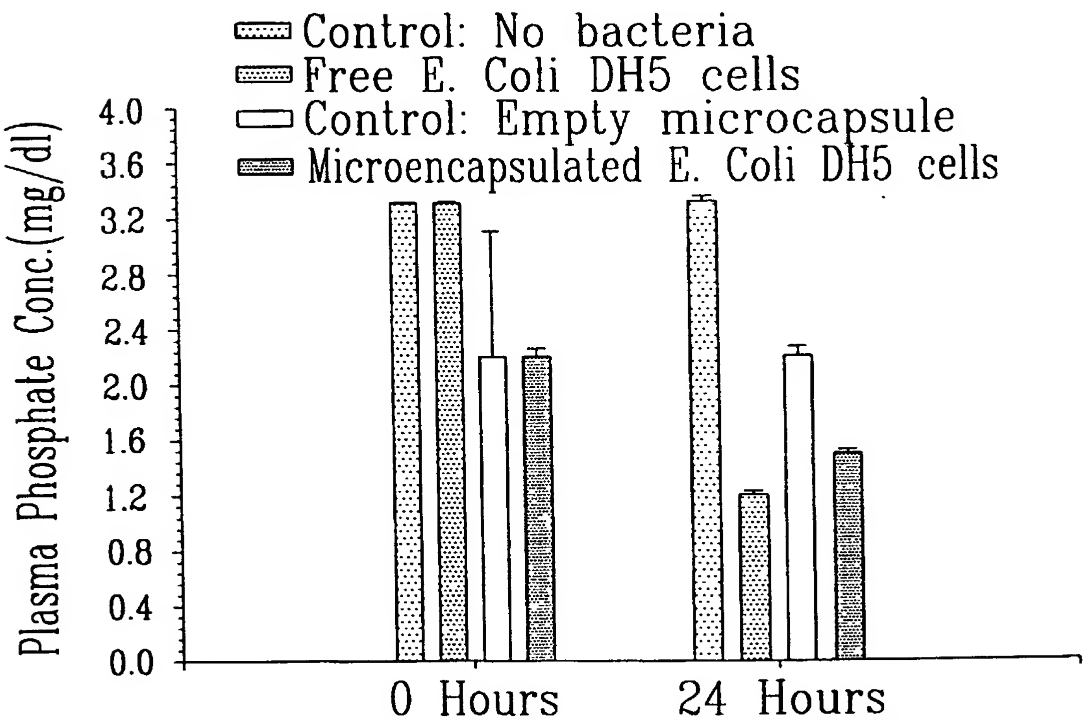
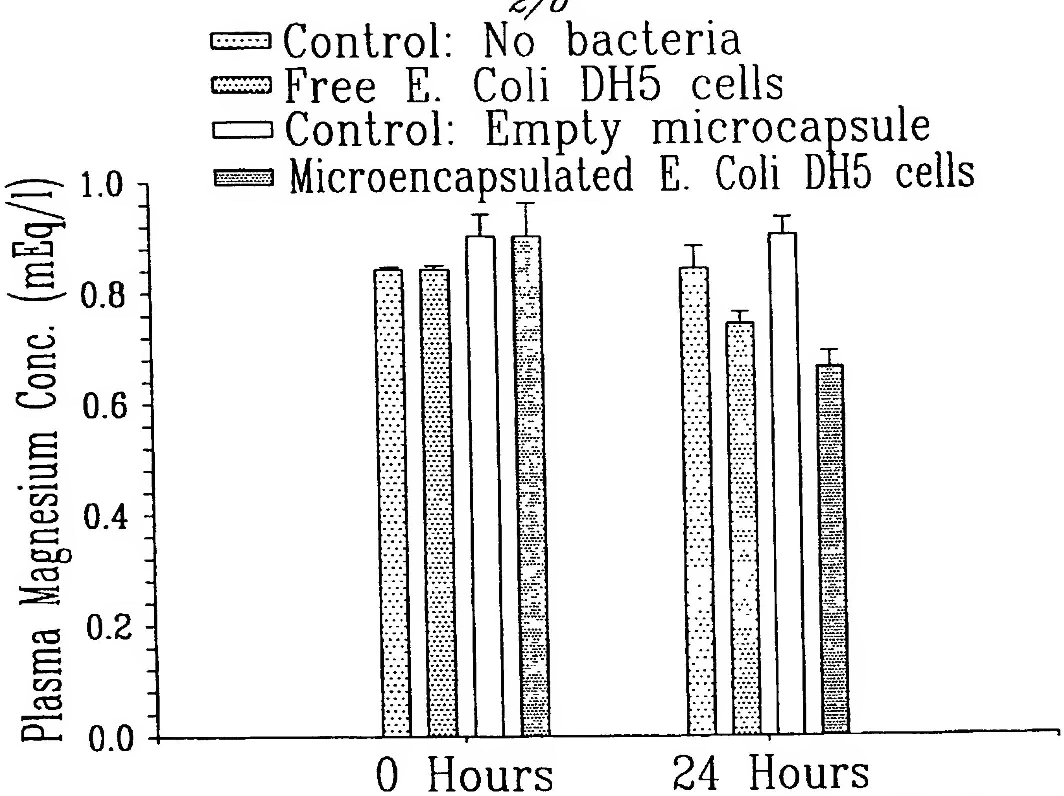
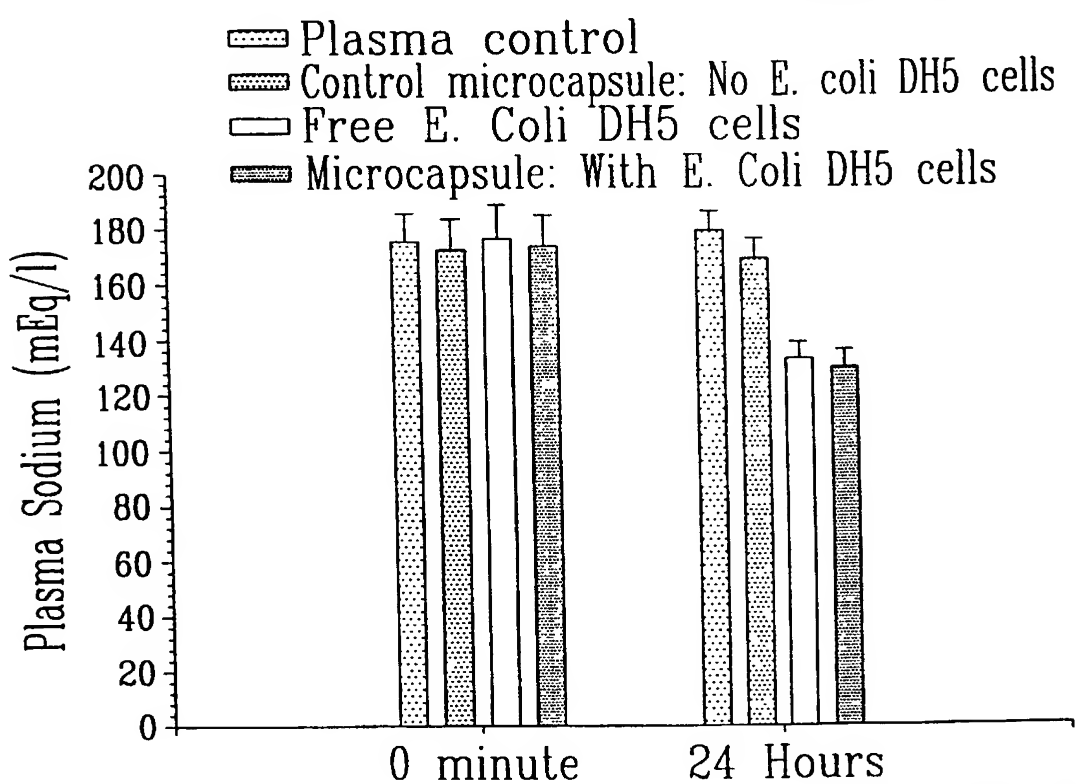

Control: No bacteria
Free E. Coli DH5 cells
Control: Empty microcapsule
Microencapsulated E. Coli DH5 cells
Plasma Phosphate Conc.(mg/dl)
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
0 Hours
24 Hours

FIG. 2



Control: No bacteria
Free E. Coli DH5 cells
Control: Empty microcapsule
Microencapsulated E. Coli DH5 cells
Plasma Magnesium Conc. (mEq/l)
1.0
0.8
0.6
0.4
0.2
0.0
0 Hours
24 Hours

FIG. 3



Plasma control
Control microcapsule: No E. coli DH5 cells
Free E. Coli DH5 cells
Microcapsule: With E. Coli DH5 cells
Plasma Sodium (mEq/l)
200
180
160
140
120
100
80
60
40
20
0
0 minute
24 Hours

FIG. 4

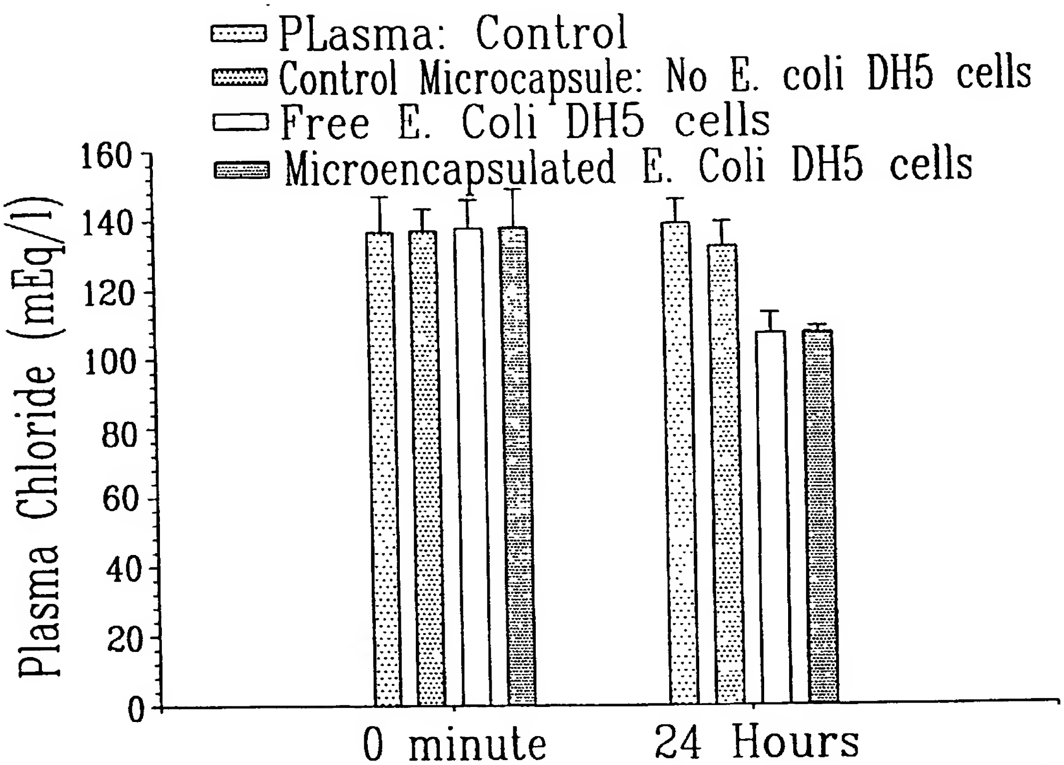

PLasma: Control
Control Microcapsule: No E. coli DH5 cells
Free E. Coli DH5 cells
Microencapsulated E. Coli DH5 cells
Plasma Chloride (mEq/l)
160
140
120
100
80
60
40
20
0
0 minute
24 Hours

FIG. 5

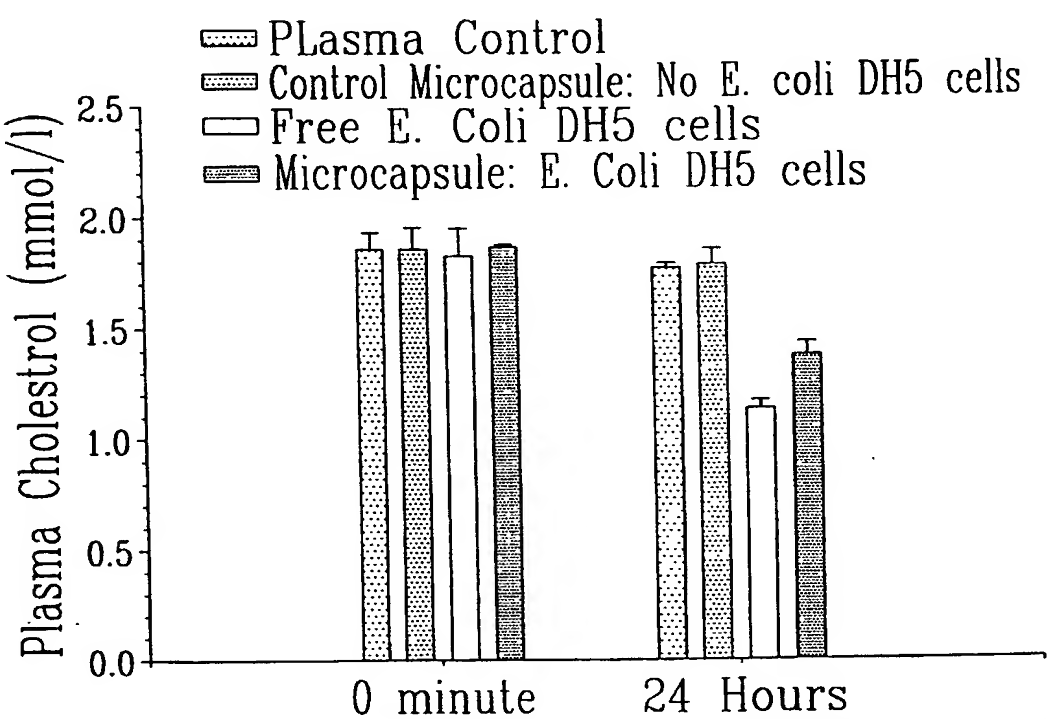

PLasma Control
Control Microcapsule: No E. coli DH5 cells
Free E. Coli DH5 cells
Microcapsule: E. Coli DH5 cells
Plasma Cholestrol (mmol/l)
2.5
2.0
1.5
1.0
0.5
0.0
0 minute
24 Hours

FIG. 6

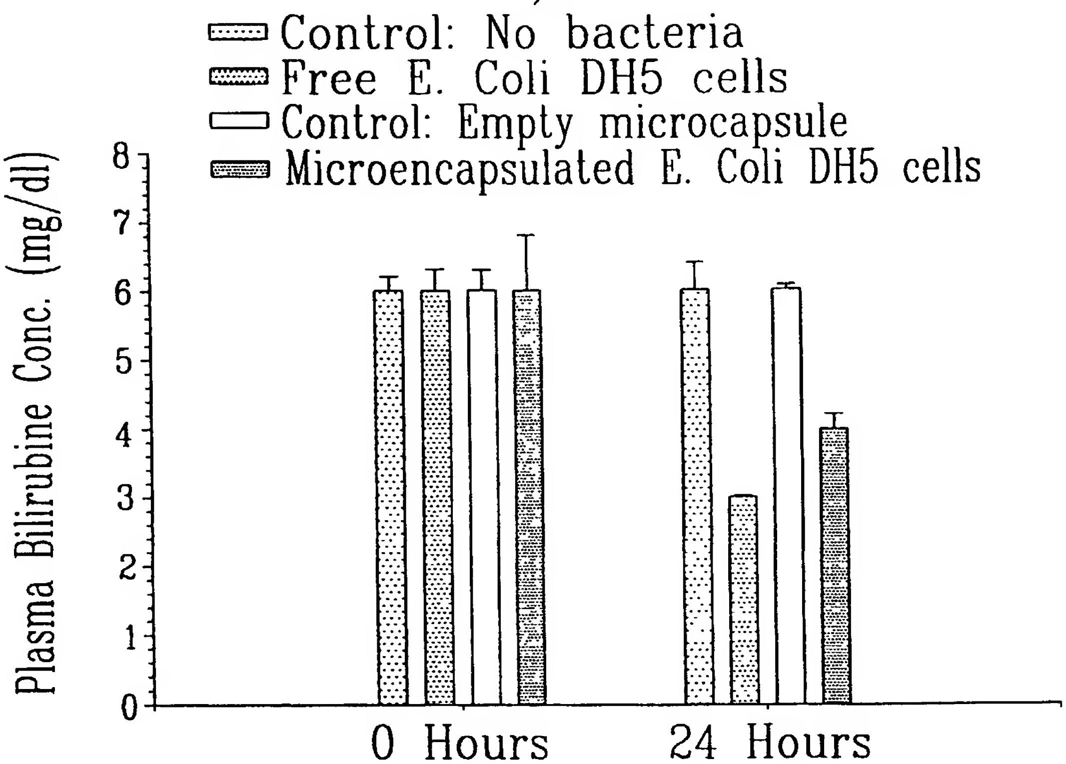

Control: No bacteria
Free E. Coli DH5 cells
Control: Empty microcapsule
Microencapsulated E. Coli DH5 cells
Plasma Bilirubine Conc. (mg/dl)
0
1
2
3
4
5
6
7
8
0 Hours
24 Hours

FIG. 7

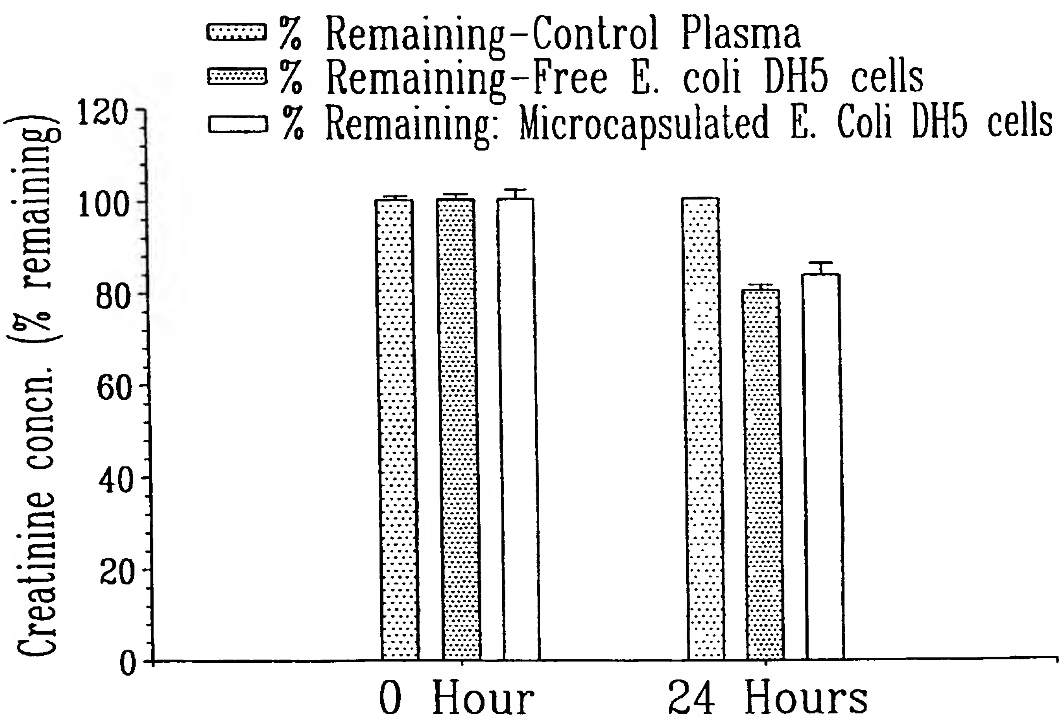

% Remaining-Control Plasma
% Remaining-Free E. coli DH5 cells
% Remaining: Microcapsulated E. Coli DH5 cells
Creatinine concn. (% remaining)
0
20
40
60
80
100
120
0 Hour
24 Hours

FIG. 8

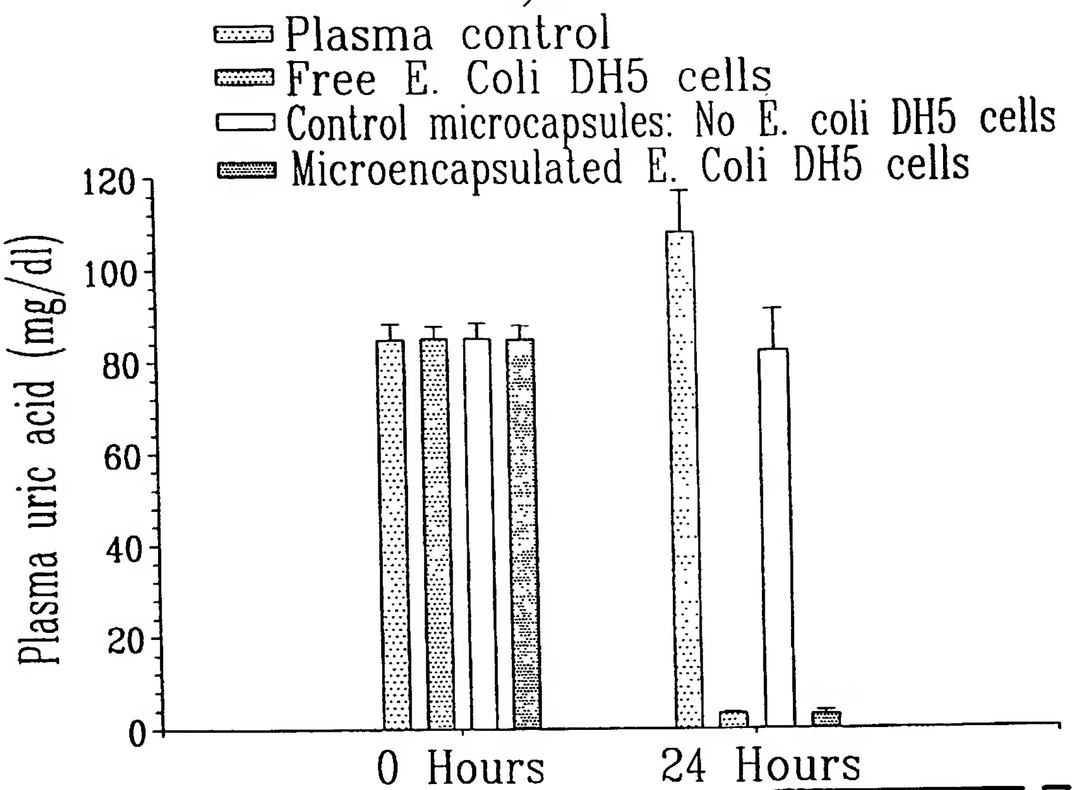

Plasma control
Free E. Coli DH5 cells
Control microcapsules: No E. coli DH5 cells
Microencapsulated E. Coli DH5 cells
Plasma uric acid (mg/dl)
120
100
80
60
40
20
0
0 Hours
24 Hours

FIG. 9

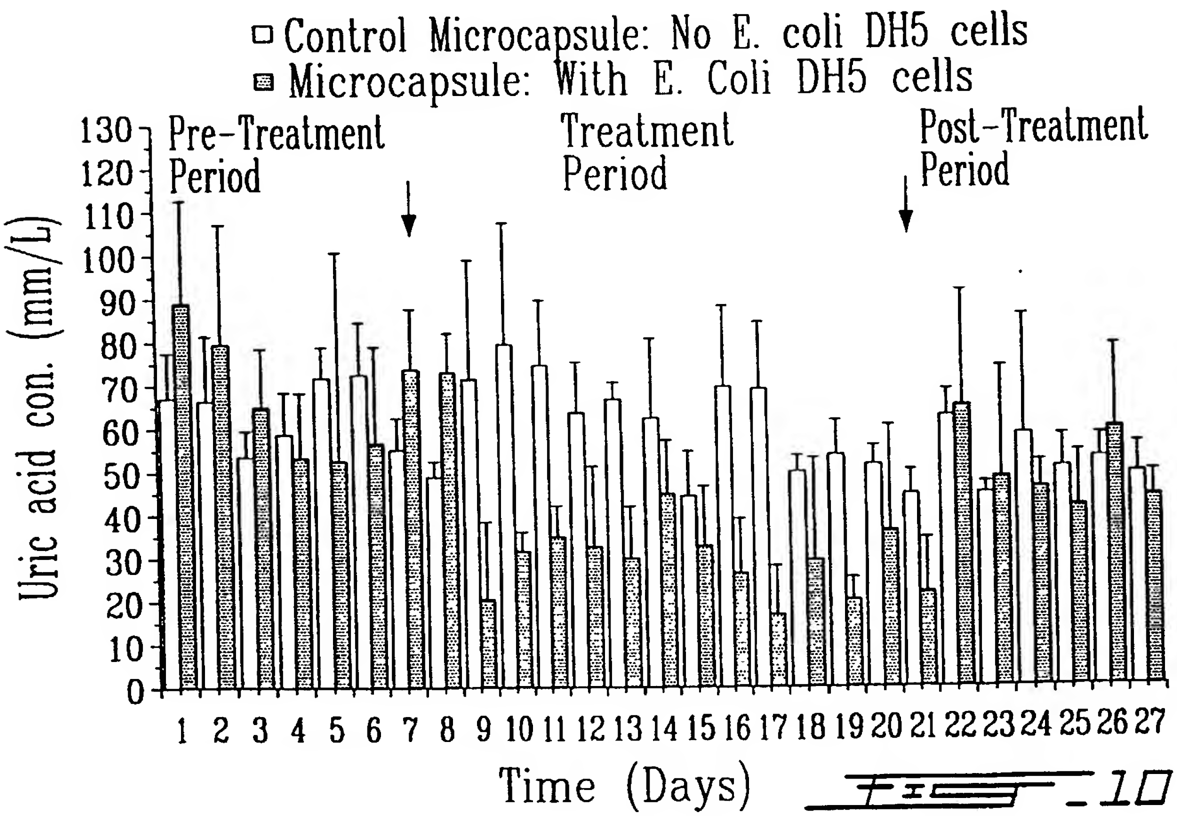

Control Microcapsule: No E. coli DH5 cells
Microcapsule: With E. Coli DH5 cells
Pre-Treatment Period
Treatment Period
Post-Treatment Period
Uric acid con. (mm/L)
130
120
110
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Time (Days)

FIG. 10

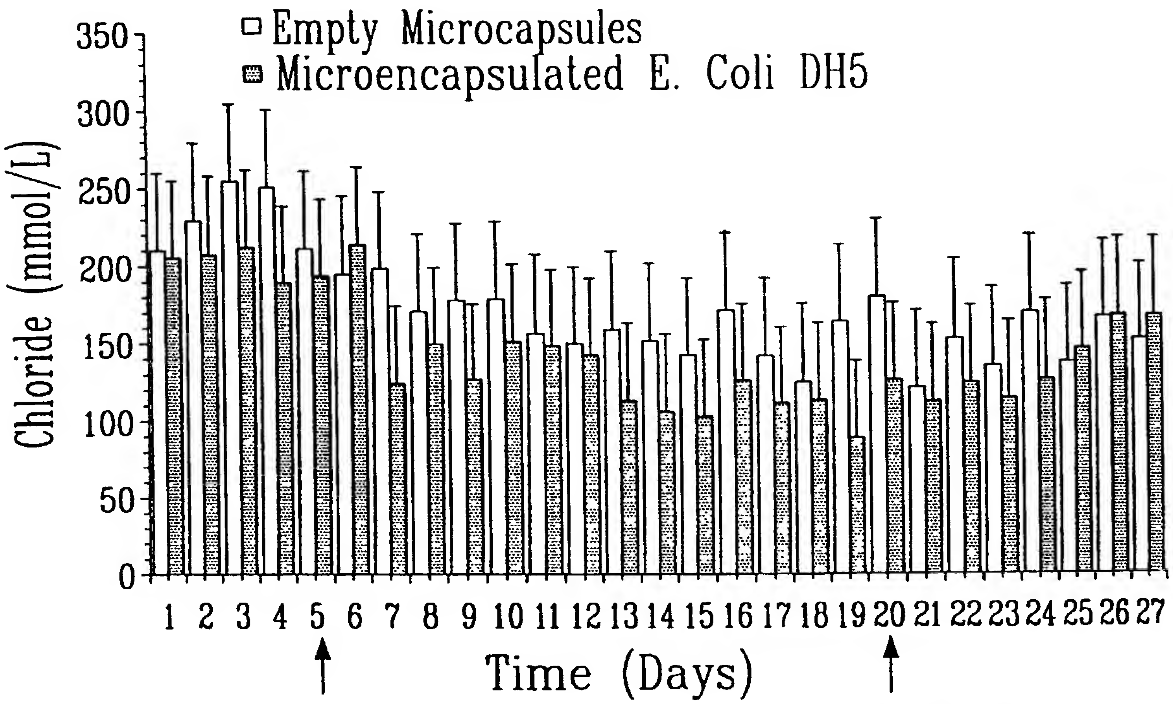

Empty Microcapsules
Microencapsulated E. Coli DH5
Chloride (mmol/L)
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Time (Days)

FIG. 11

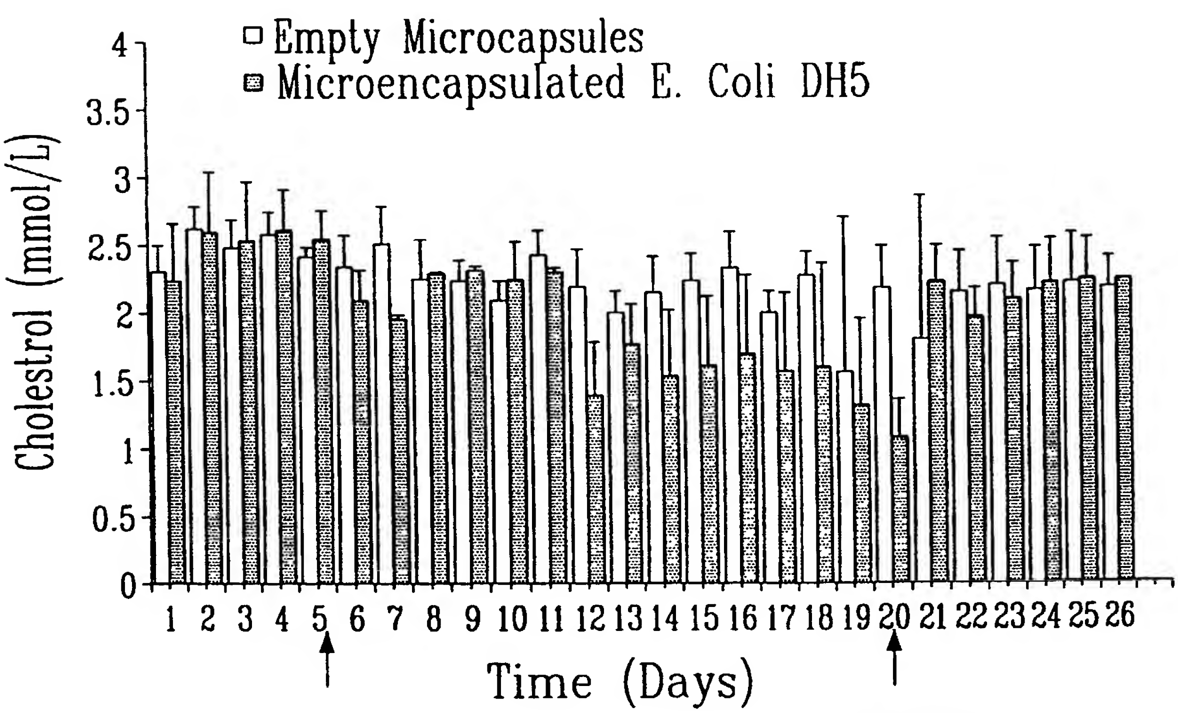

Empty Microcapsules
Microencapsulated E. Coli DH5
Cholestrol (mmol/L)
4
3.5
3
2.5
2
1.5
1
0.5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
Time (Days)

FIG. 12

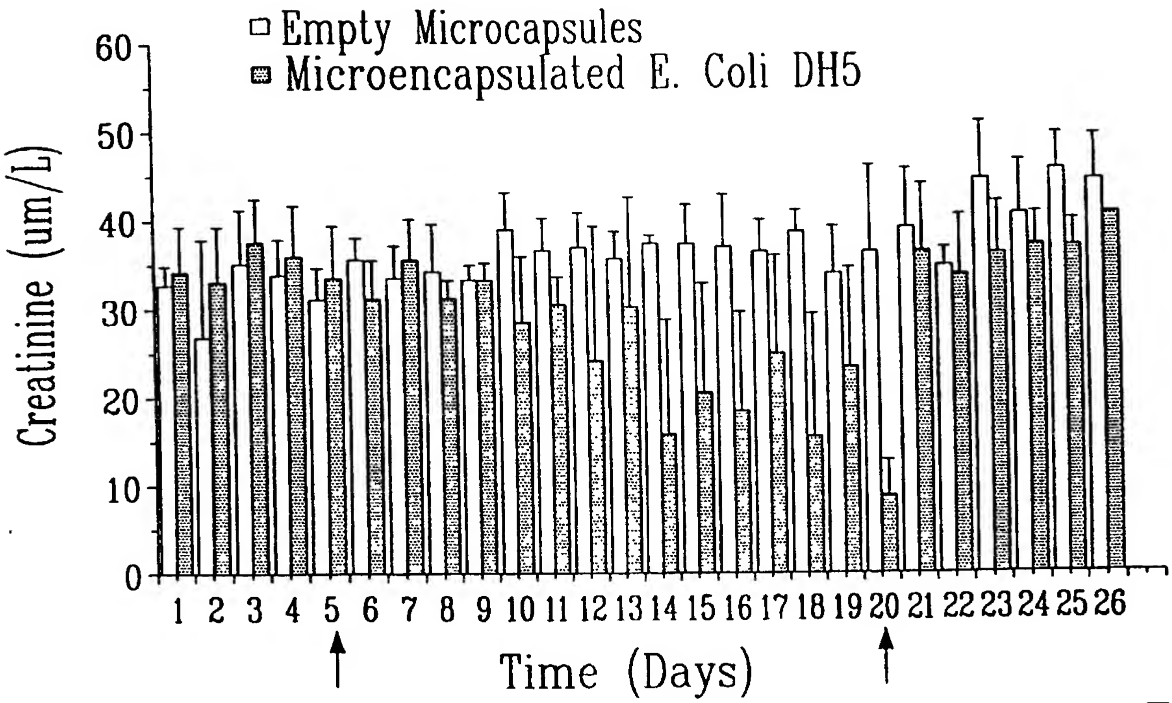

Empty Microcapsules
Microencapsulated E. Coli DH5
Creatinine (um/L)
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
Time (Days)

FIG. 13

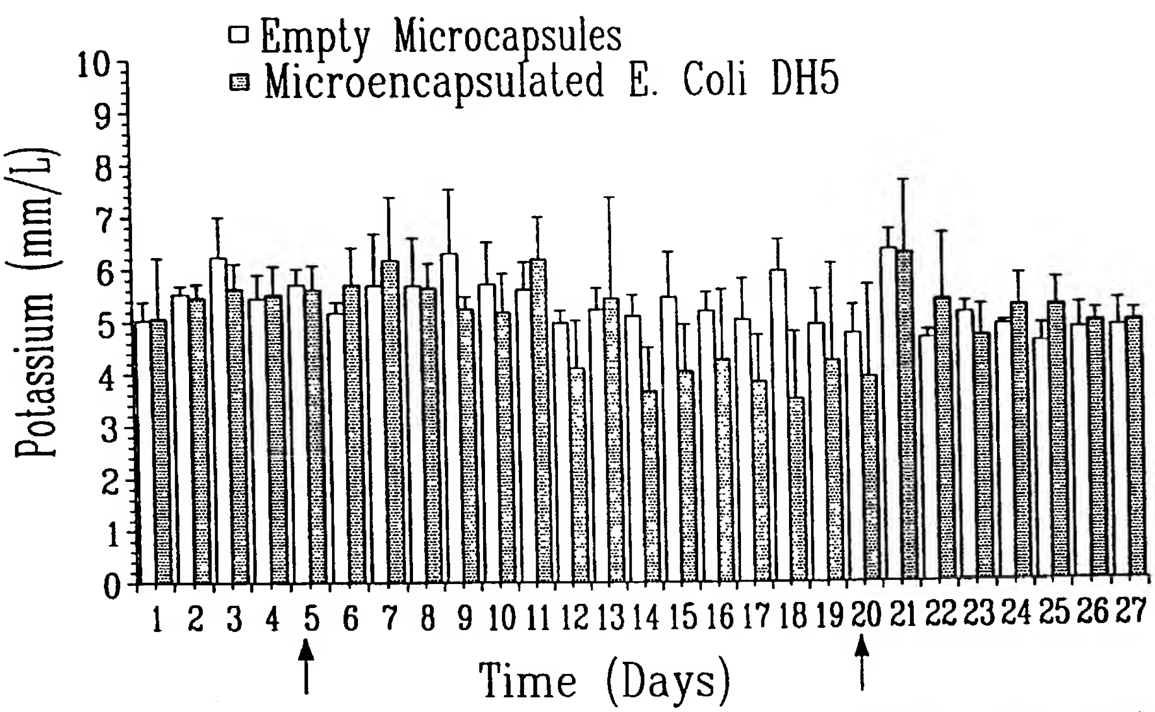

Empty Microcapsules
Microencapsulated E. Coli DH5
Potassium (mm/L)
10
9
8
7
6
5
4
3
2
1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Time (Days)

FIG. 14

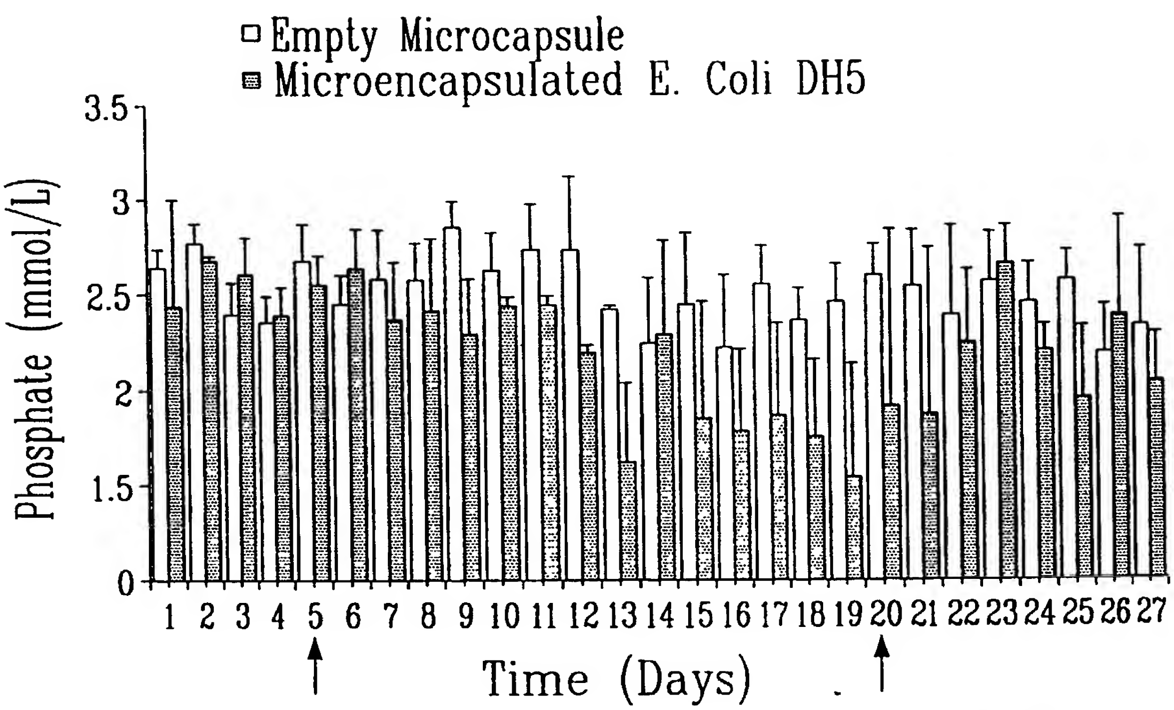

Empty Microcapsule
Microencapsulated E. Coli DH5
Phosphate (mmol/L)
3.5
3
2.5
2
1.5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Time (Days)

FIG. 15

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DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that: My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed at 201) below or an original, first and joint inventor (if plural names are listed at 201-208 below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

MICROENCAPSULATED GENETICALLY ENGINEERED E. COLI DH 5 CELLS FOR THE REMOVAL OF UNDESIRED ELECTROLYTES AND/OR METABOLITES

which is described and claimed in:

☐ the specification attached hereto.

☐ the specification in U.S. Application Serial Number__________________, filed on ____________________;

☒ the specification in PCT International Application Number PCT/CA/00842 filed on 27 April 2000 and amended on 25 May 2001;

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a). I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign/PCT Applications and Any Priority Claims Under 35 U.S.C. §119:			
Application No.	Filing Date	Country	Priority Claimed under 35 U.S.C. §119?
PCT/CA00/00482	27 April 2000 (27.04.00)	Canada	☒YES ☐NO
			☐YES ☐NO
			☐YES ☐NO
			☐YES ☐NO

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT International application(s) designating the United States of America that is/are listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose material information as defined in 37 CFR §1 56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

Prior U.S. Applications or PCT International Applications Designating the U.S-Benefit under 35 U.S.C. §120				
U.S. Applications		Status (Check One)		
Application Serial No.	U.S. Filing Date	Patented	Pending	Abandoned

PCT Applications Designating the U.S.					
Application No.	Filing Date	U.S. Serial No. Assigned			

CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)
(35 U.S.C. §119(e))

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Applicants	Provisional Application Number	**Filing Date**
Satya Prakash and Tomas M. S. Chang	60/131,468	28 April 1999 (28.04.99)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) with full powers of association, substitution and revocation to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY AND ZIP CODE

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signature of Inventor 201 (PRAKASH) Satya Prakash	Date: 24/05/2002
Signature of Inventor 202 (CHANG) T.M.S. Chang	Date: 28/05/2002
Signature of Inventor 203	Date: